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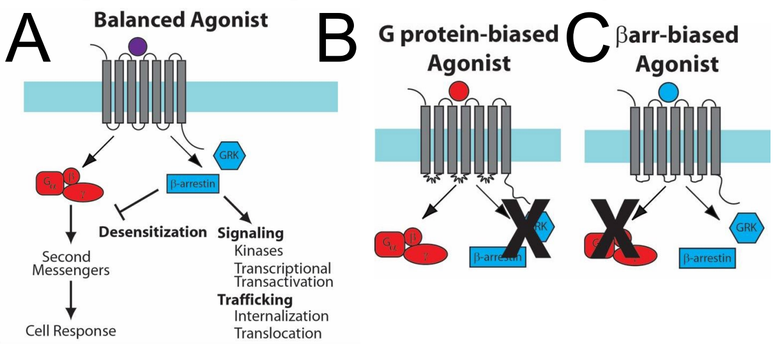
Introduction

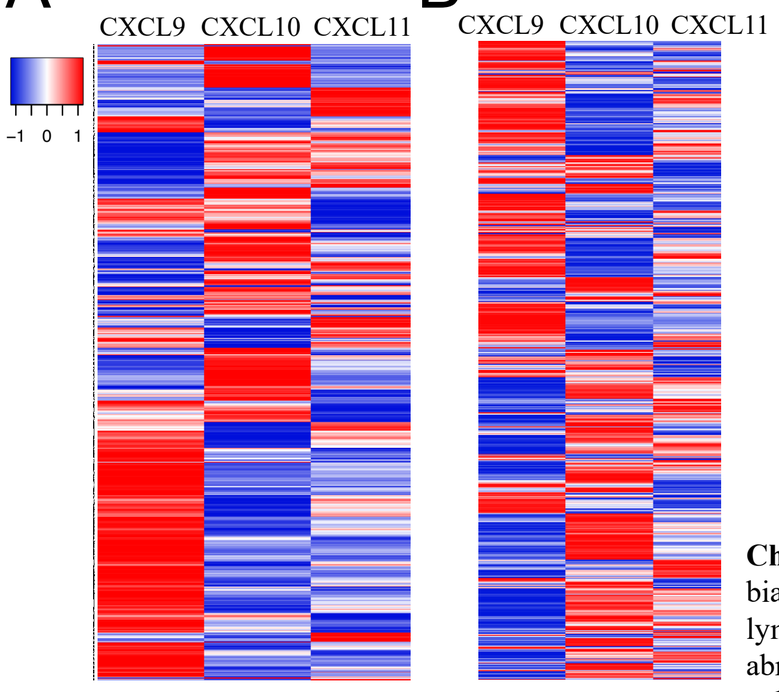
Biased agonism, the ability of different ligands for the same receptor to selectively activate some signaling pathways while blocking others, is now an established paradigm for GPCR signaling. Panel A demonstrates a 'balanced' agonist, signaling through both G protein as well as β-arrestin pathways. Panel B shows a pure 'G protein biased' agonist, while panel C shows a pure 'β-arrestin biased' agonist.



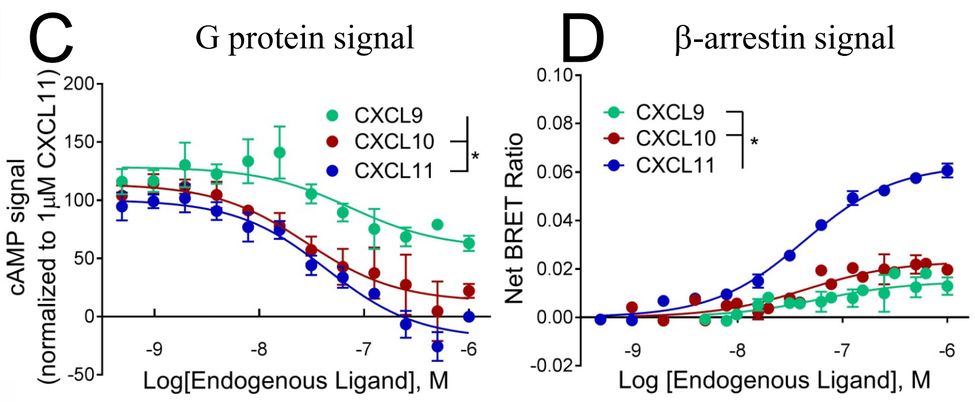
Objectives and Methods

CXCR3 is an important chemokine receptor that regulates T cell-mediated inflammation but has no FDA approved drugs. The goal of this study was to measure biased signaling at CXCR3 and assess the therapeutic potential of selectively targeting certain CXCR3 signaling pathways with biased agonists. Biased signaling was measured through transcriptomic and phosphoproteomic analyses, as well as multidimensional cellular signaling assays. Small molecule biased agonists for G protein and β-arrestin were identified. Inflammatory properties of the biased agonists were tested in a mouse model of T cell allergy.

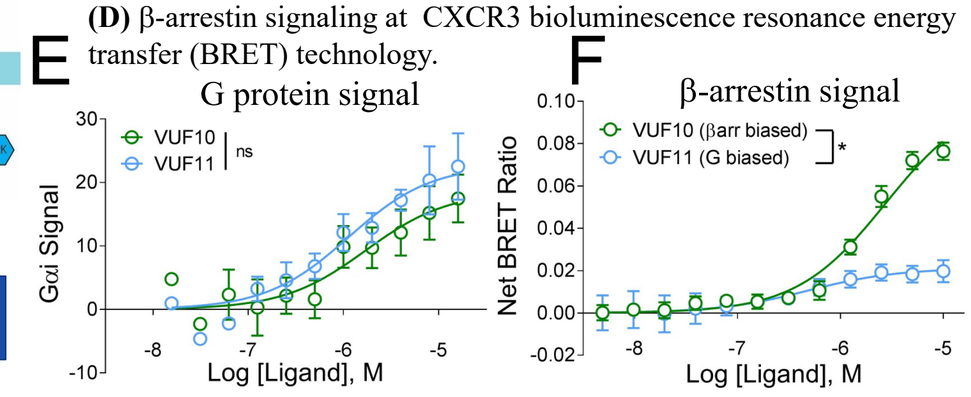
Results: Endogenous CXCR3 chemokines are biased

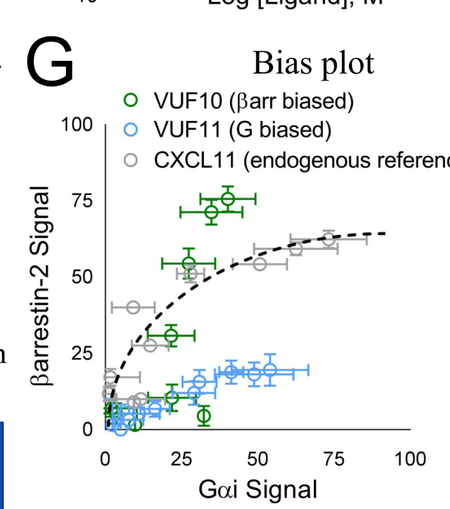


CXCR3 panomics. Cellular **(A)** RNA seq and **(B)** phosphoprotein clusters in log(2) scale following chemokine stimulation relative to vehicle treatment.

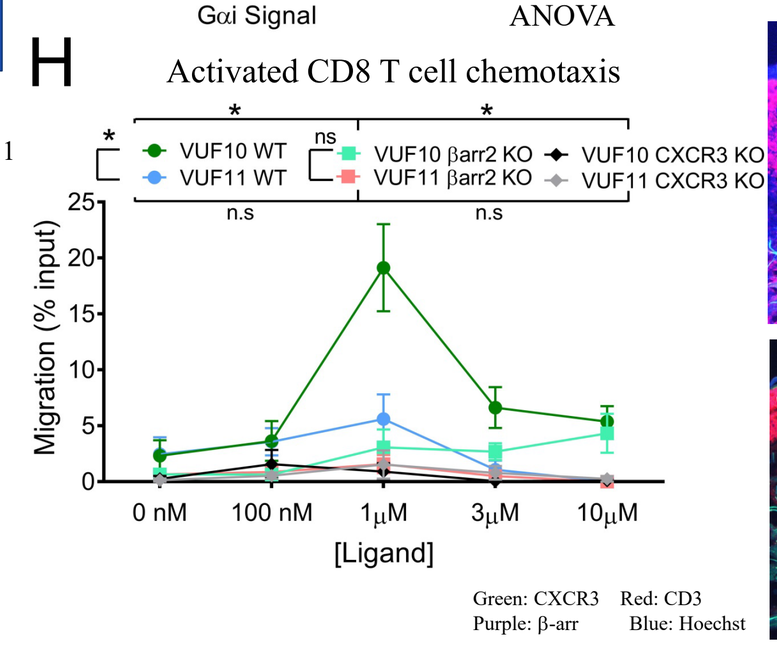


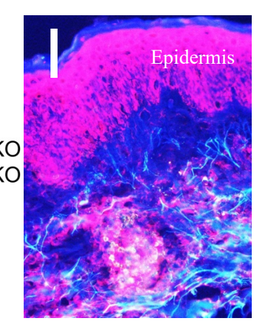
(C) Gαi protein signaling assessed through cAMP inhibition at CXCR3. **(D)** β-arrestin signaling at CXCR3 bioluminescence resonance energy transfer (BRET) technology.

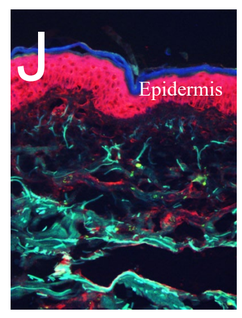




Identification of synthetic biased agonists. Small molecule CXCR3 ligands were assessed for **(E)** G protein activity or **(F)** β-arrestin recruitment. **(G)** VUF10 was identified as a β-arrestin-biased agonist, while VUF11 was identified as a G protein-biased agonist relative to CXCL11, a full agonist at both G protein and β-arrestin pathways. Both agonists have the same affinity for CXCR3. *p<0.05 by two-way ANOVA







Green: CXCR3 Red: CD3
Purple: β-arr Blue: Hoechst

Chemotactic response of T cells to CXCR3 biased ligands: (H) A β-arrestin biased agonist caused significantly greater migration of activated CD8+CD44+ lymphocytes isolated from WT mice compared to a G-biased agonist, which was abrogated in β-arrestin-2 KO and absent in CXCR3 KO lymphocytes. CXCR3+T cells from patients displayed a similar pattern of chemotaxis (data not shown). Colocalization of CXCR3, β-arr, and CD3 was observed in patients with **(I)** patch test (+) but not **(J)** (-) skin biopsies for T cell allergy. *p<0.05 by two-way ANOVA

Results: CXCR3 biased agonists differentially regulate inflammation

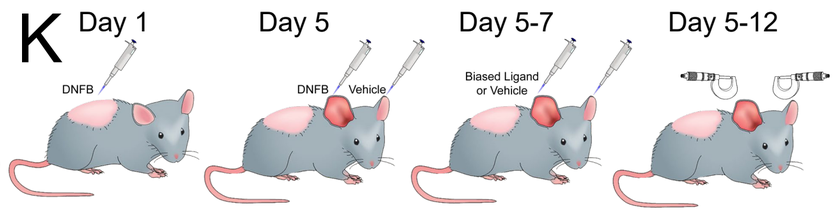
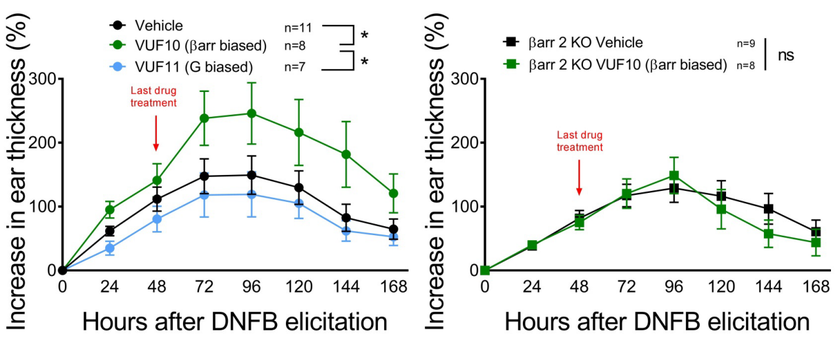


Image adapted from Parham *et al* 2006.

(K) Timeline of T cell inflammation model. Mice were topically treated with the allergen DNFB (0.5% DNFB sensitization on the back, 0.3% elicitation on the ears) and CXCR3 ligands at the indicated time points. Change in ear thickness was measured.



β-arrestin-biased agonist potentiates inflammation in a T cell contact hypersensitivity allergy model: (L) Wild-type mice topically treated with a β-arrestin-biased agonist, but not a G-protein-biased agonist, had a potentiated inflammatory response in a mouse model of T cell allergy. No potentiation of inflammation by the βarr-biased agonist was observed in either **(M)** β-arrestin-2 KO mice (p=0.77) or CXCR3 KO mice (not shown). *p<0.05 by two-way ANOVA

Conclusions

- Endogenous CXCR3 chemokines (CXCL9, CXCL10, and CXCL11) are not redundant and induce vastly different cellular signaling events.
- Distinct CXCR3 signaling events appear to be physiologically relevant, as a CXCR3 β-arrestin-biased agonist increased chemotaxis and T cell-mediated inflammation relative to a CXCR3 G-biased agonist.
- When designing drugs targeting chemokine receptors, looking beyond affinity and G protein signaling pathways will likely be necessary for a desired therapeutic benefit.

Acknowledgements

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